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Evaluation of Soybean [Glycine max (L.) Merr.] F₁ Hybrids

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Heterosis is an important factor in development of hybrid cultivars. Few heterosis studies have been done in soybean [Glycine max (L.) Merr.]. This is because manual cross-pollination is difficult and time-consuming, and not economical to produce large quantities of hybrid seed. Male-sterility systems identified in soybean, combined with insect-mediated cross-pollination, have been shown to produce large quantities of hybrid seed. This procedure was used in this study to produce hybrid seed for replicated yield trials with the objectives to: 1) evaluate the agronomic performance of soybean F_1 hybrids; and 2) estimate heterosis for yield and other agronomic traits of the F_1 hybrids. Parental genotypes were two male-sterile, female-fertile lines with the ms3 and ms9 mutations, and a group of six high-yielding male-parent lines. The experiment was conducted in two years at several locations. In 2005, twelve F_1 hybrids were evaluated along with parent lines. Midparent heterosis (MPH) ranged from -29% to +32%, and high-parent

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heterosis (HPH) from -23% to +1%. In 2006, eleven hybrids were evaluated. MPH values ranged from -53% to -21%, and HPH from -66% to -35%. Seed size and seed protein content showed HPH for some combinations. For traits related to vegetative growth, such as height and lodging, positive MPH and HPH were observed. In general, depending on the year and parent combinations, there were hybrids that performed better than the mid-parent values, suggesting that heterosis was identified in soybean. Significant differences for yield between hybrids of the ms3 and ms9 groups also were observed.

KEYWORDS Soybean, Glycine max, hybrids, heterosis, male-sterility, insect-mediated cross-pollination, agronomic performance, yield

INTRODUCTION

Heterosis, the measure of the average superiority of an F_1 hybrid over its parental inbred lines, is an important factor in the development of hybrid cultivars. Heterosis can be expressed when the parents of a hybrid have different alleles at a locus and there is some level of dominance or epistasis among the alleles (Falconer & Mackay, 1996).

Heterosis does exist in soybean [Glycine max (L.) Merr.] and, if appropriate parent combinations and an economical means of producing hybrid seed could be identified, yield increases would benefit soybean growers. Reports in the literature have indicated that in some cases, the best hybrids have yielded +10% to +20% more than the higher-yielding parent (Palmer et al., 2001). A summary of fourteen heterosis reports since 1930, using a total of 456 different crosses, has shown that the average value of midparent heterosis (MPH) ranged from +14% to +46%, and average value for HPH ranged from +4% to +34% (Palmer et al., 2001). Most of the studies, however, were done with space-planted hybrid plants, and only a few hybrid combinations were tested. For this reason, results of these experiments cannot be extrapolated to commercial plantings.

In other experiments, where more hybrid seed was available, yield tests were done in three-row plots with at least three replications. Average yield MPH percentages for twenty-seven and seven hybrid combinations were +3% and +4%, respectively (Nelson & Bernard, 1984; Lewers, 1996). Sun et al. (1999) in a summary of data collected from a comprehensive heterosis test program in China reported that 846 of 1123 combinations showed positive MPH. Of the 846 combinations, 248 showed a mean HPH of +20%. Hybrid seed was obtained by hand pollination and the evaluation of heterosis was done at six research institutes in China in single-row plots with two replications.

Ortiz-Perez et al. (2007) evaluated heterosis for yield and agronomic traits from single crosses, three-way crosses, and backcrosses (BC₁F₁). MPH values for yield ranged from -59% to +37% for single crosses, -14% to +16% for three-way crosses, and -7% to +42% for BC₁F₁ HPH range was from -66% to +17% for single crosses, -25% to -5% for three-way crosses, and -16% to +42% for BC₁F₁ crosses. Burton and Brownie (2006) evaluated the F₁ generation of two combinations derived from crosses between current soybean cultivars. The average yield of one cross was +16 % greater than the highest-yielding parent; for the other cross it was +5 % greater than the highest-yielding parent. These results suggest that significant yield increases are possible for some combinations, but release of commercial hybrids, at least in the United States, remains a challenge because several aspects of the hybrid-seed-production scheme need to be evaluated. One important aspect is to find a feasible system of F₁ hybrid-seed production. It is important to note that in China, however, the first hybrid soybean cultivar was released in 2002 (Palmer et al, 2003).

The work that we report here has several unique aspects compared with previous studies in which heterosis was measured. First, our study used an efficient insect-mediated, cross-pollination hybrid-seed-production system. During the course of the work conducted by Ortiz-Perez et al. (2008), the authors selected within female-parent genotypes for insect attractiveness, using an increased number of out-crossed pods as a selection criterion. With this selection criterion, the authors observed an increase in the amount of hybrid seed produced. Based on these results, we only used female plants derived from the most productive individuals to maximize hybrid-seed production. Another unique aspect of our work that differed from that of Ortiz-Perez et al. (2007) was that the male parents used for hybrid-seed production were current high-yielding public cultivars as opposed to the old public cultivars. A third aspect different from previous studies was that we conducted replicated field tests using four-row plots. Many of the studies in hybrid soybean had been conducted using spaced single plants (Palmer et al., 2001), single-row plots (Sun et al., 1999), and three-row plots (Nelson & Bernard, 1984; Lewers, 1996), because of scarcity of hybrid seed. This was not the case in the current study. The objectives of our study were, therefore, to: 1) evaluate agronomic performance of soybean F₁ hybrids, and 2) estimate heterosis for yield and other agronomic characteristics of the F_1 hybrids.

MATERIALS AND METHODS

Twelve F_1 hybrids were developed by crossing two nuclear male-sterile lines as female to six male parents (Table 1). For each female genotype, the homozygous dominant male-fertile, female-fertile sibling was planted as

TABLE 1 Soybean Hybrids and Parents Evaluated in 2005 and 2006 at Two Locations Near Ames, IA (Loc 1 and Loc 2), and One Location Near Gilbert, IA (Loc 3)

		2005		20	006
Genotype	Loc 1	Loc 2	Loc 3	Loc 1	Loc 2
Female parents ^a					
Ms3Ms3	X^{b}	X	X	\mathbf{X}	X
Ms9Ms9	X	X	X	X	
Male parents					
IA2050	X	X	X	X	X
IA2052	X	X	X	X	X
K1547	X	X	X	X	X
LG00-6182	X	X	X	X	X
LG00-6193	X	X	X	X	X
LG01-4756	X	X	X	X	X
Hybrids					
ms3 × IA2050	X	X		X	X
$ms3 \times IA2052$	X			X	X
ms3 × K1547	X			X	
$ms3 \times LG00-6182$	X	X		X	
$ms3 \times LG00-6193$	X	X		X	X
$ms3 \times LG01-4756$	X			X	X
$ms9 \times IA2050$	X	X	X	X	
$ms9 \times IA2052$	X	X	X		
ms9 × K1547	X	X	X	X	
$ms9 \times LG00-6182$	X	X		X	
$ms9 \times LG00-6193$	X	X		X	
ms9 × LG01–4756	X	X		X	

^aFertile sibling of male-sterile, female-fertile parents.

control in the same field along with hybrids and the six male parents. In every yield trial, a total of 20 genotypes was evaluated.

Plant Materials

Female parents had excellent insect pollinator attraction determined on the basis of out-crossed pod production per plant. These lines had been selected by Ortiz-Perez et al. (2008) in a previous study. Lines segregating for nuclear male-sterile mutations were used as female parents: *ms3ms3* (T284H; Chaudhari & Davis, 1977) and *ms9ms9* (T359H; Palmer, 2000). The six male parent lines included three high-yielding public cultivars—K1547 (Kansas State University, Manhattan, Kan.), IA2052 (Iowa State University, Ames, Iowa)—and three high-yielding public breeding lines with greater than 50% exotic germplasm in them, LG00–6182, LG00–6193, and LG01–4756 from USDA-ARS at

^bX = Genotype planted at that location

Urbana, Ill. Breeding lines LG00–6182 and LG00–6193 were selections from the same two-parent mating. Male parents were chosen because of high yield, adequate plant height, and maturity adapted to Iowa. Twelve $\rm F_1$ hybrid combinations were developed by insect-mediated cross-pollination between two female lines crossed to each of the six male lines.

F₁ Seed Production

Hybrid seed was produced in a full-season nursery in Chile, South America. For hybrid-seed production, each plot had six rows. Rows one and six were planted with the male parent; rows two to five were planted with the segregating male-sterile line. Rows were spaced 76 cm apart and were 4.8 m long, with 1.2 m spacing between plots. Planting density was 14 seeds/m. Each plot was replicated three times for each of the twelve hybrid combinations in a randomized complete-block design, where the two male-sterile, female-fertile lines (ms3ms3 and ms9ms9) were crossed with each of the six selected males.

At flowering, male-sterile plants were identified and labeled, and fertile siblings were removed. This procedure was done in the middle rows (2–5) where the segregating male-sterile lines were planted for each hybrid combination. Insect vectors transferred pollen from the male-parent rows to the male-sterile, female-fertile plants. Alfalfa leaf cutter bees (*Megachile rotundata* F.) were used as the insect pollinator species. Each plot was bulk-harvested, and hybrid seed for each combination was sent to Iowa for planting in summers of 2005 and 2006 (Table 1).

Field Testing

Four-row plots of F₁ hybrids and parents were planted in replicated tests. Each row was 5.2 m long with a space of 0.76 m between rows. Two locations near Ames and one location near Gilbert, Iowa, were used for the experiment. In 2005, parental lines were evaluated at all locations; hybrid combinations also were evaluated, although not at all locations (Table 1). In 2006, parental lines, except the homozygous dominant male-fertile, femalefertile *Ms9Ms9*, were evaluated at two locations near Ames, Iowa. In 2006, the hybrid combination *ms9* x IA2052 was not evaluated because of lack of seed; the other hybrid combinations were evaluated, although not at all locations due to non-availability of sufficient seed. A randomized complete-block design with two replications was used at each location and in each year. Because all hybrids were not evaluated at all locations, the design was unbalanced.

Traits evaluated were seed yield, seed protein and oil content, lodging, plant height, maturity date, and seed size. Maturity date was recorded as the number of days from planting until 95% of the pods in the two middle rows

were brown (stage R8) (Fehr et al., 1971). Plant height and lodging were recorded at harvest. Plant height was measured in centimeters on two plants from each of the two middle rows. Lodging was a visual observation of the whole plot, recorded on a 1 to 5 scale, with 1 being all plants upright, 3 being plants at 45°, and 5 being all plants prostrate. The two middle rows were harvested at maturity and the seed was used to determine yield, seed size, and seed composition. Samples of 25 g were sent to the USDA National Center for Agricultural Utilization Research (NCAUR), Peoria, Ill., to determine seed protein and seed oil content via near-infrared transmittance.

Statistical Analysis

For all genotypes, a mixed model analysis of variance (ANOVA) was performed separately for each year on the data for each agronomic trait. Because of the unbalanced design, the data were analyzed using PROC MIXED of SAS v. 9.1 (SAS Institute, 2003). In the model, genotype was a fixed effect and location, genotype by location and replications within location were considered random effects. Least square means (LSMEANS) were used to estimate the hybrid and parent performance each year.

Hybrid performance relative to parents can be measured as mid-parent heterosis and high-parent heterosis (Fehr, 1991). Mid-parent heterosis was determined as:

MPH (%) = $[(F_1 - MP)/MP] \times 100$, where F_1 = performance of the hybrid and MP = mean performance of the parents

High-parent heterosis was determined as:

HPH (%) = $[(F_1 - HP)/HP] \times 100$, where F_1 = performance of hybrid and HP = performance of 'high' or best parent

The ESTIMATE statement of SAS v. 9.1 (SAS Institute, 2003) was used to determine the difference between the mean of each hybrid with the mean of its parents. The LSMEANS statement with a PDIFF option, which allows differences of the LS-means to be displayed, was used to estimate the difference between the hybrid and the parent with the best performance for a specific trait. Multiple-comparison tests were done with the Bonferroni method. The BON option of SAS v. 9.1 (SAS Institute, 2003) was used to estimate adjusted p-values for multiple comparisons. To determine the correlation between variables, Pearson correlation coefficients were computed using the PROC CORR statement of SAS v.9.1 (SAS Institute, 2003).

RESULTS

In 2005, the analysis of variance for the single-cross populations and parents indicated significant differences among genotypes for all traits (Table 2). In addition, orthogonal contrasts were performed to detect specific differences between groups of genotypes (male parents, female parents, and single crosses). In general, and considering both years and all traits, the contrasts between parents and hybrids showed significant (P < 0.05) differences between groups of genotypes (data not shown). There was a significant interaction between genotypes and locations for all traits, except seed protein and oil content. This indicated that protein and oil content across genotypes were similar at both locations in 2005. In 2006, the analysis of variance for the single-cross populations and parents indicated significant differences among genotypes (Table 2). A significant interaction between genotypes and locations was observed only for seed protein content. The differences between the two years could be attributed to genotypes planted at fewer locations in 2006 (Table 1). All significant differences were at the P < 0.05 level.

Agronomic Performance of Parent Lines and Hybrids

Male parents had the best yield performance among all groups in both years (Table 3). Average yield of male parents was 2,797 kg/ha in 2005 and 3,297 kg/ha in 2006. In 2005, the highest yielding male parent was IA2050, but LG01–4756 was the highest yielding male parent in 2006. Mean yield of female parents was 1,897 kg/ha in 2005 and 1,696 kg/ha in 2006, which were significantly lower than the yield of the male parents in respective years. Among female parents, the homozygous-dominant, male-fertile, female-fertile *Ms9Ms9* was the highest yielding in both years, although the difference was not significant.

The mean yield of hybrids of the ms3 group was 2,418 kg/ha in 2005 and 1,262 kg/ha in 2006 (Table 3). In this group, the highest yielding hybrid was $ms3 \times K1547$ in 2005 and $ms3 \times IA2050$ in 2006. In the ms9 group, the mean yield was 1,920 kg/ha in 2005 and 1,903 kg/ha in 2006. The highest yielding hybrid was $ms9 \times IA2052$ in 2005 and $ms9 \times IG01$ –4756 in 2006.

Hybrids of the *ms9* group had mean seed size larger than that of parents in both years (Table 4). Female parents and hybrids had the highest content of seed protein and oil content that was lower than that of male parents. Hybrids with high seed protein content had low yields, and the correlation between the two traits was negative (Table 5).

In both years, mean maturity of female parents and hybrids was later than that of male parents. In general and on average, hybrids were taller than their parents, particularly in 2006. Lodging was similar among parents and hybrids in 2005, although hybrids had higher lodging scores than their parents in 2006 (Table 4).

TABLE 2 Mean Squares for Yield, Seed Size, Seed Protein Content, Seed Oil Content, Maturity, Height, and Lodging for Soybean Parents and F₁ Hybrids in 2005 and 2006 at Ames and Gilbert, IA

		Height	376.8*	151.5	69.2	9.6	62.5
		Lodging	2.1*	1.7	0.2	0.4	0.4
	ures	Maturity Lodging Height	49.8*	130	10	27.7	17.3
2006	Mean squares	Seed		1.8		0.2	0.4
2	Me	Seed Seed size protein	7.2*	16.3*	2.8*	1.1	1.0
		Seed size	6.2*	1.4	0.4	0.1	0.4
		Yield	18 2819594*	440631	82608	54718	89803
	•	dfa	18	Π	11	7	26
		Height	293.7*	289.3	41.9*	62.9*	27.9
		Lodging	0.7*	4.4	0.5*	1.1*	0.2
	es	Seed oil Maturity Lodging Height df ^a	31.1*	576.7*	11.6^{*}	19.8*	6.7
2005	Mean squares	Seed	2.2*	8.6*	0.5	0.3	0.5
20	Меа	Seed Seed protein oil	5.14*	60.5*	1.38	9.0	1.9
		Seed size		1.06		0.5	1.7
		df ^a Yield	1161470^*	16754211*	199381*	832564*	47 158030
	'	dfa	19	7	56	ϵ	47
		Source of variation	Genotype	Location	Location* 26 199381*	genotype Replication (location)	Residual

^aDifferent degrees of freedom for each year because all genotypes were not planted at all locations every year.

^{*}P-value ≤ 0.05 .

TABLE 3 Yield of Parents and Hybrids. Mid-Parent Heterosis (MPH), High-Parent Heterosis (HPH), and Mean Heterosis for Each *ms* Hybrid Group in 2005 and 2006, in Replicated Tests Averaged Across Locations^a

		2005			2006	
Genotype	Yield(Kg ha ⁻¹)	MPH(%)	HPH(%)	Yield(Kg ha ⁻¹)	MPH(%)	HPH (%)
Female parents ^b						
Ms3Ms3	1504			1459		
Ms9Ms9	2290			1932		
Mean	1897			1696		
Male parents						
IA2050	3142			3149		
IA2052	2805			3447		
K1547	2813			3391		
LG00-6182	2399			3073		
LG00-6193	2913			3105		
LG01-4756	2710			3616		
Mean	2797			3297		
Hybrids						
ms3 × IA2050	2433	+5	-23	1410	-39*	-55*
$ms3 \times IA2052$	2840	+32	+1	1155	-53*	-66*
ms3 × K1547	2849	+32	+1	1137	-53*	-66*
ms3 × LG00–6182	2139	+10	-11	1279	-44*	-58*
ms3 × LG00–6193	1662	-25	-43	1223	-46*	-61*
ms3 × LG01–4756	2587	+23	- 5	1370	-40*	-62*
Mean	2418	+13	-13	1262	-46	-62
$ms9 \times IA2050$	1948	-28*	-38*	1840	-28	-42
$ms9 \times IA2052$	2049	-20	-27	ND^{c}	ND	ND
$ms9 \times K1547$	2027	-21*	-28	1636	-39*	-52*
$ms9 \times LG00-6182$	1817	-22	-24	1985	-21	-35
ms9 × LG00–6193	1904	-27*	-35	1932	-23	-38
ms9 × LG01–4756	1775	-29*	-35	2120	-24	-4 1
Mean	1920	-24	-31	1903	-27	-42

^aTwo replications at two locations near Ames, and one location near Gilbert, IA in 2005; two replications near Ames, IA in 2006.

Heterosis in Hybrids

Hybrids of the ms3 group had positive MPH for yield in 2005, with the exception of one combination (Table 3). In 2005, mean MPH value of the ms3 group was +13%. In this group, the hybrids with the highest values were $ms3 \times IA2052$ and $ms3 \times K1547$, both showing +32% MPH. Also in 2005, mean MPH for hybrids of the ms9 group was -24%. In 2006, yield MPH with both female parents was negative and ranged from -53% to -21% (Table 3). The trend in high positive values of MPH for hybrids of the ms3 group observed in 2005 and the negative values observed in 2006 may

^bFertile siblings of male-sterile, female-fertile parents.

^cND = No data.

^{*}P-value ≤ 0.05 .

TABLE 4 Seed Size, Seed Protein Content, Seed Oil Content, Height, Lodging, and Maturity of F₁ Hybrids and Parents in 2005 and 2006, in Replicated Tests Averaged Across Locations^a

	Seed (mg se	l size eed ⁻¹)	Seed protein (g Kg ⁻¹)	srotein g^{-1}	Seed oil $(g \text{ Kg}^{-1})$	d oil (g ⁻¹)	Heigh	Height (cm)	Lodging ^b (score)	ging ^b rre)	Maturity ^c (days)	ırity ^c ys)
Genotype	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Female parents ^d												
Ms3Ms3	133	124	428	432	183	163	76	111	2.8	3.3	138	146
05M65M	154	159	420	440	187	164	91	88	1.7	2.6	133	142
Mean	143	141	424	436	185	164	94	100	2.2	2.9	136	144
Male parents												
IA2050	152	156	405	410	199	191	9/	81	1.5	1.5	129	139
IA2052	141	143	408	424	201	188	88	66	2.2	1.8	132	138
K1547	148	149	394	396	206	183	78	68	1.3	1.0	136	144
LG00-6182	144	147	407	398	192	182	102	116	1.8	2.3	133	146
LG00-6193	166	168	412	409	190	180	105	120	2.0	3.0	134	143
LG01-4756	162	166	410	406	199	174	92	86	2.0	2.0	138	144
Mean	152	155	406	407	198	183	06	101	1.8	1.9	134	142

$ms3 \times IA2050$ 149 135 409 428 191 165 98 110 1.8 3.0 135 148 $ms3 \times IA2052$ 146 139 408 438 195 158 101 112 2.0 3.3 129 159 159 159 150 <th>Hybrids</th> <th></th>	Hybrids												
146 139 408 438 195 158 101 112 2.0 3.3 129 144 138 405 435 194 160 103 108 3.0 3.1 130 146 149 428 436 185 161 98 115 2.3 3.1 130 143 142 429 440 184 157 98 106 2.3 3.3 134 143 140 418 434 187 159 103 114 2.3 3.5 134 171 183 425 435 189 160 100 2.3 4.1 133 165 ND 425 ND 186 ND 94 ND 1.8 ND 133 185 165 426 435 187 167 89 104 1.9 3.1 133 163 172 418 190 177 101 111 2.2 3.1 134 162	2050	149	135	409	428	191	165	86	110	1.8	3.0	135	148
144 138 405 435 194 160 103 108 3.0 3.1 130 146 149 428 436 185 161 98 115 2.3 3.1 139 143 142 429 440 184 157 98 106 2.3 3.8 135 143 140 418 434 187 159 103 114 2.3 3.5 134 154 141 416 435 189 160 100 111 2.3 3.3 134 171 183 425 435 185 166 96 109 2.3 4.1 133 165 ND 425 ND 186 ND 94 ND 1.8 ND 133 185 165 426 435 187 167 89 104 1.9 3.1 133 163 172 418 418 190 177 101 111 2.2 3.1 134	2052	146	139	408	438	195	158	101	112	2.0	3.3	129	150
146 149 428 436 185 161 98 115 2.3 3.1 139 143 142 429 440 184 157 98 106 2.3 3.8 135 143 140 418 434 187 159 103 114 2.3 3.5 134 145 141 416 435 189 160 100 1.3 3.3 134 155 ND 425 ND 186 ND 94 ND° 1.8 ND 133 165 ND 425 187 167 89 104 1.9 3.1 133 185 172 418 190 177 101 111 2.2 3.1 135 162 172 425 431 190 166 93 107 2.2 3.1 134 163 172 425 431 180	1547	144	138	405	435	194	160	103	108	3.0	3.1	130	136
143 142 429 440 184 157 98 106 2.3 3.8 135 143 140 418 434 187 159 103 114 2.3 3.5 134 145 141 416 435 189 160 100 111 2.3 3.3 134 171 183 425 435 185 166 96 109 2.3 4.1 133 185 165 ND 186 ND 94 ND° 1.8 ND 133 185 165 426 435 187 167 89 104 1.9 3.1 133 163 172 418 190 177 101 111 2.2 3.1 135 162 172 425 431 190 166 93 107 2.2 3.1 134 169 172 423 427 188 172 94 107 2.0 3.1 134	300-6182	146	149	428	436	185	161	86	115	2.3	3.1	139	149
143 140 418 434 187 159 103 114 2.3 3.5 134 145 141 416 435 189 160 100 111 2.3 3.3 134 171 183 425 435 185 166 96 109 2.3 4.1 133 165 ND 426 435 187 167 89 104 1.9 3.1 133 163 172 418 418 190 177 101 111 2.2 3.1 135 162 172 425 431 190 166 93 107 2.2 3.1 135 162 172 423 427 188 172 94 107 2.0 3.1 134	G00 - 6193	143	142	429	440	184	157	86	106	2.3	3.8	135	149
145 141 416 435 189 160 100 111 2.3 3.3 134 171 183 425 435 185 166 96 109 2.3 4.1 133 165 ND 425 ND 186 ND 94 ND° 1.8 ND 133 185 165 426 435 187 167 89 104 1.9 3.1 133 163 172 418 190 177 101 111 2.2 3.1 135 171 170 422 414 189 181 92 103 1.8 2.1 134 162 172 425 427 188 172 94 107 2.0 3.1 134	301–4756	143	140	418	434	187	159	103	114	2.3	3.5	134	149
171 183 425 435 185 166 96 109 2.3 4.1 133 165 ND 425 ND 186 ND 94 ND° 1.8 ND 133 185 165 426 435 187 167 89 104 1.9 3.1 133 82 163 172 418 418 190 177 101 111 2.2 3.1 135 93 171 170 422 434 189 181 92 103 1.8 2.1 134 56 162 172 425 431 190 166 93 107 2.2 3.1 134 169 172 423 427 188 172 94 107 2.0 3.1 134		145	141	416	435	189	160	100	1111	2.3	3.3	134	146
165 ND 425 ND 186 ND 94 ND° 1.8 ND 133 82 165 426 435 187 167 89 104 1.9 3.1 133 82 163 172 418 418 190 177 101 111 2.2 3.1 135 93 171 170 422 444 189 181 92 103 1.8 2.1 134 56 162 172 425 431 190 166 93 107 2.2 3.1 135 169 172 423 427 188 172 94 107 2.0 3.1 134	A 2050	171	183	425	435	185	166	96	109	2.3	4.1	133	148
82 163 172 418 418 190 177 101 111 2.2 3.1 133 83 171 170 422 414 189 181 92 103 1.8 2.1 134 56 162 172 423 427 188 172 94 107 2.0 3.1 135	X2052	165	ND	425	ND	186	ND	94	$ND_{\rm e}$	1.8	ND	133	S
163 172 418 418 190 177 101 111 2.2 3.1 135 171 170 422 414 189 181 92 103 1.8 2.1 134 162 172 425 431 190 166 93 107 2.2 3.1 135 169 172 423 427 188 172 94 107 2.0 3.1 134	1547	185	165	426	435	187	167	68	104	1.9	3.1	133	147
171 170 422 414 189 181 92 103 1.8 2.1 134 162 172 425 431 190 166 93 107 2.2 3.1 135 169 172 423 427 188 172 94 107 2.0 3.1 134	G00–6182	163	172	418	418	190	177	101	1111	2.2	3.1	135	148
162 172 425 431 190 166 93 107 2.2 3.1 135 169 172 423 427 188 172 94 107 2.0 3.1 134	G00 - 6193	171	170	422	414	189	181	92	103	1.8	2.1	134	148
169 172 423 427 188 172 94 107 2.0 3.1 134	G01-4756	162	172	425	431	190	166	93	107	2.2	3.1	135	148
		169	172	423	427	188	172	94	107	2.0	3.1	134	148

^aTwo replications at two locations near Ames, and one location near Gilbert, IA in 2005; two replications near Ames, IA in 2006.
^bLodging score: 1 = upright, 5 = prostrate.

^cMaturity = days from planting to stage R8.

^dFertile siblings of male-sterile, female-fertile parents.

^eND = No data.

TABLE 5 Correlation Matrix for Yield, Seed Size, Seed Protein Content, Seed Oil Content, Maturity, Height, and Lodging in 2005 at Ames and Gilbert, IA

	Seed size Yield	0.36 1
		0-1
	Seed protein	-0.86^{**} 0.47^{**}
2006	Seed oil	0.78** -0.35* -0.90**
	Maturity	-0.52 0.09 0.35* -0.28
	Height	-0.36 0.19* 0.55* -0.69** 1
	Lodging	-0.31* -0.08 0.41 -0.55 0.12* 0.73**
	Height Lodging	-0.46** 0.07 0.05** -0.60** 0.32* 0.62**
		-0.40** 0.24 0.59** -0.63** 0.57**
	Seed oil Maturity	-0.57** 0.33 0.44** -0.61**
2005	Seed oil	0.84** -0.02 -0.84**
	Seed protein	-0.75** 0.15
	Yield Seed size	0.07
	Yield	1
		Yield Seed size Seed protein Seed oil Maturity Height Lodging

*P-value ≤ 0.05 . **P-value ≤ 0.01 .

be explained in part by the poor performance of the female parent lines in 2006.

In 2005, the two hybrids with the highest positive MPH for yield also showed positive HPH, although the estimates were not significantly different from zero (Table 3). In general, all HPH values were negative or not different from zero, irrespective of the female parent used in the hybrid combination. The mean HPH value for hybrids of the ms3 group was -13% and -31% for hybrids of the ms9 group. Similar results were observed in 2006; mean HPH for hybrids of the ms3 group was -62% and -42% for hybrids of the ms9 group (Table 3).

For seed size in 2005, MPH had mostly positive and HPH had some positive values (Table 6). In 2006, no consistent trend was observed in both heterosis estimates. In general, and considering both years, MPH ranged from –4% to +23% and HPH ranged from –15% to +21%. Similar variable results were observed for seed protein content heterosis estimates (Table 6). For this trait in 2005, hybrids of the *ms9* group had a mean MPH of +3% and HPH of +1%. In 2006, hybrids of the *ms3* group had a mean MPH of +4% and HPH of +1%. For seed oil content, the majority of the hybrids had negative MPH and HPH estimates.

In this study, hybrids that had negative MPH and HPH values for yield also had negative values for oil content (Tables 3 and 6). In both years, yield and oil content had a positive association (r = 0.78 in 2005, and r = 0.84 in 2006; both significant at P < 0.05) (Table 5).

For maturity, lodging, and plant height, hybrids expressed varying levels of heterosis depending on the year (Table 6). In some cases, trait values were higher than those of their parents, which are reflected in positive estimates of MPH and HPH. In other cases, values were negative. For maturity, considering both years and both female parents, MPH ranged from -6% to +6% and HPH ranged from -7% to +4%. For lodging, MPH ranged from -24% to +101% and HPH ranged from -29% to +58%. For plant height, MPH ranged from -8% to +28% and HPH ranged from -14% to +23%. In both years, lodging and height had a positive association (r = 0.73 in 2005, and r = 0.62 in 2006; significant at P < 0.05).

DISCUSSION

Estimates of MPH and HPH were, in general, variable across years, locations, and parent combinations. Depending on the year and parent combinations, there were hybrids that performed better than the mean performance of parents (mid-parent value), although none of the hybrids was better than the best parent of the cross. In general, however, male-parent lines had yield performances that were superior to those of hybrids. A possible explanation for this is that the homozygous dominant male-fertile, female-fertile

TABLE 6 Mid-Parent Heterosis (MPH), High-Parent Heterosis (HPH), and Mean Heterosis for Each *ms* Hybrid Group for Seed Size, Seed Protein Content, Seed Oil Content, Height, Lodging, and Maturity in 2005 and 2006, in Replicated Tests Averaged Across Locations^a

		Seed	Seed Size			Seed Protein	rotein			Seed Oil	Oil			Height	ght			Lodging	ing			Maturity	rity	
	20	2005	2006	90	2005	92	2006	90	2005	5	2006	90	2005	5	2006	9.	2005	<i>i</i> C	2006	90	2005	51	2006	9
Hybrids	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	(%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)
$ms3 \times IA2050$	+5	-2	-3	-13*	-2	5	+2	-1	0	4	-7-	-14	+14*	+1	+14*	-1	-16	-35	+24*	6-	+1	-3	*44*	+1
$ms3 \times IA2052$	<u>+</u>	+4	+	-3	-2	ή.	+2	+	+1	5	-10^{*}	-16°	6+	+4	9+	0	-18	-27	+30*	0	5	1	*5	43
$ms3 \times K1547$	+2	-3	+1		7	4	*5+	0	0	9	*&	-13	+17*	9+	8	-3	+51*	6+	+47*	4	*	9	*9	<u></u>
$ms3 \times LG00-6182 +5$	+	+2	+10*	+2	+2	0	*4	+1	-1	4	•9	-12	T	4	+1	1	0	-17	+111*	9	+2	0	+2	+2
$ms3 \times LG00-6193$	-4	-14	-3	-15*	+2	0	4	+2	-2	-3	*6	-13	-3	<u></u>	8	-11	-3	-17	+20*	+15	7	-2	+3*	+2
$ms3 \times LG01-4756$ -3	-3	-12	4	-15*	0	-2	+3	0	-2	9	*_/	8-	6+	9+	-2	+2	-5	-18	+12*	8+	-3	-3	+3*	+2
Mean	+2	4	+1	6-	0	5	+4	+1	-1	4	φ	-13	+	+1	+3	-2	+1	-17	+24	+1	-2	-3	+2	0
$ms9 \times IA2050$	+12*	+11	+16*	+15	43	+	+2	-1	*4	*	*9	-13	+15*	÷ ~	+28	+23	*444	+34	+101*	+58	+2	0	*9+	4
$ms9 \times IA2052$	+12*	8+	ND	R	+3	+1	$ND_{\rm p}$	R	*4	*∞	ND	S	5+	+3	S	N	9-	-15	S	ND	+1	0	ND^{y}	S
$ms9 \times K1547$	+23*	+21*	+	+4	**	+	4	1	5	*6	4	9	÷	-2	+17	+17	+28	+10	+73*	+20	T	-2	+3	+2
$ms9 \times \text{LG006182}$	6+	9+	+13*	8+	+1	0	0	5	0	-1	+2	-3	+5	T	8+	5	+21	+18	+28	+19	+2	7	+3	+1
$ms9 \times LG00-6193 +7$	+	+3	+v4	+	+2	+1	-2	9	0	0	÷	0	9	-13	ī	-14	-4	-10	-24	-29	0	0	+4	+4
$ms9 \times LG01-4756 + 2$	+2	0	9+	+4	+2	+1	+2	-2	-2	5	-2	4	+2	+1	+15	6+	+16	8+	+35	+19	T	-5	+3	+2
Mean	+11	8+	6+	9+	+3	+1	+	-3	-5	5	-	9	+4	T	+14	9+	+16	· - -	+43	+17	0	0	4	+3
																								I

*P-value ≤ 0.05 .

^a Two replications at two locations near Ames, and one location near Gilbert, IA in 2005; two replications near Ames, IA in 2006.

 b ND = No data.

siblings of each ms mutant had poor yield performance. The male-sterile alleles were introgressed into old genetic lines, in which yield was not considered a prime factor for selection. The low-yielding background of the female lines may have had a negative effect on F₁ hybrid performance and on heterosis expression. It is possible that if the male-sterile genes used in this study had been introgressed into modern higher-yielding genetic backgrounds, hybrids would have had better agronomic performance and consequently positive values for heterosis. Burton and Brownie (2006) suggested that possible genetic bases for heterosis in soybean could be gene complementation, linked dominant alleles that are inherited as a unit, a greater number of dominant alleles in the F₁ than in the parents, and multiple dosage-dependant regulatory loci and/or overdominance. Using these assumptions, they stated, "Heterosis should be predictive of good parental combinations." This observation suggests that once the male-sterile genes are introgressed into higher-yielding genetic backgrounds, it would be important to evaluate a large number of hybrid combinations using the highest-vielding cultivars as parents.

Two female lines with different male-sterile alleles were used as parents, which allowed evaluation of performance of male-sterile sources in hybrid combinations. A result of the comparison was finding significant differences for yield between some hybrids of the *ms3* group and the *ms9* group, even when they had the same male parent. It is, however, important to indicate that the male-sterile alleles used in the study were introgressed into different genetic backgrounds and this may partly explain the distinct results. Because soybean does have numerous different alleles for male sterility (Palmer et al., 2004), it would be important in future studies to include several of the alleles introgressed into the same genetic background. In the study reported herein, two interpretations are possible: 1) an interaction between male-sterile alleles with their respective genetic background; or 2) differences in the interactions between parental combinations e.g., specific combining ability.

Variability in hybrid performance and of estimates for MPH and HPH observed in the study were nevertheless similar to previous reports. Heterosis estimates for yield in studies conducted with spaced plants were reported by Weber, Empig, and Thorne (1970) who evaluated 85 crosses with a mean HPH of +13% and a mean MPH of +25%. Chaudhari and Singh (1974) observed mean HPH for seed yield of +26%. Heterosis for yield in several studies done under standard plant density has been reported. Cerna et al. (1997) found that HPH values of 16 F_1 crosses ranged from -17% to +97% and MPH ranged from +7% to +102%. In the study of Manjarrez-Sandoval et al. (1997), HPH ranged from +1% to +15% in twenty-four hybrid combinations; mean MPH was +7%. In the study of Nelson and Bernard (1984), HPH from five of twenty-seven hybrid combinations ranged from +13% to +19% and mean MPH was +8%. In 1996, Lewers found that the mean HPH

for thirty-six testcrosses was +7%. In more recent studies, Pandini, Natal, and Celis de Almeida Lopes (2002) evaluated thirty F_1 hybrids, which had HPH ranging from -44% to +72% and MPH from -6% to +132%. Burton and Brownie (2006) reported a mean HPH of +16% and +5% in two crosses. In the study of Ortiz-Perez et al. (2007), HPH in single crosses varied from -41% to +11%, and MPH varied from -34% to +15%. In only a few of these studies, male-sterile systems were used to obtain hybrid seed. This, in itself, is a difference that needs to be considered when comparing heterosis estimates across studies.

Significant positive MPH and HPH for plant height, lodging, seed size, and seed protein content were found in several crosses. The observations for plant height and lodging indicated that heterosis was present mainly for traits associated with vegetative growth. These observations are in agreement with published information (Nelson & Bernard, 1984). According to Lewers et al. (1998), it is possible that vegetative heterosis may increase early lodging and pod abortion and, in turn, reduce grain yield.

An increase in seed size is commonly observed for F₁ plants normally grown in soybean crossing blocks (S. Cianzio and R. Palmer, personal communication). A similar pattern of heterosis was found in our study for this trait. The positive heterosis observed for seed protein content might be the result of the inverse relationship widely reported in the literature between seed yield and seed protein content and seed protein and seed oil content. Negative correlations between seed protein and seed oil and between seed protein and yield were also detected in this study and by Burton (1987). Different genotypes and maturity groups were used in the two studies, however.

In soybean, most gene action reported for economically important traits is additive, and heritability estimates are low (Brim & Cockerham, 1961; Burton, 1987). In the current study, deviations from the mid-parent value were found for yield, seed size, seed protein content, seed oil content, maturity, height, and lodging, which may indicate epistatic effects, as suggested by Thorne and Fehr (1970).

In soybean, heterosis effects are not yet well understood and several explanations have been proposed by Burton and Brownie (2006); for example, "i) gene complementation or interaction of duplicate favorable loci in repulsion linkage, ii) linked dominant alleles inherited as a unit, iii) a greater number of dominant alleles in the F₁ than in either parent separately, iv) multiple dosage-dependant regulatory loci, v) and/or overdominance." If heterosis in soybean is governed by any of the above-mentioned mechanisms, it is necessary that complementarity exist between both parent lines to obtain yield heterosis in the hybrid; in other words, both parents must possess dominant genes at different loci controlling seed yield (Pandini, Natal, and Celis de Almeida Lopes, 2002).

In the current study, a limited number of combinations was evaluated and results cannot be extrapolated to other crosses; however, an insight into the complexities and difficulties about the evaluation of heterosis and the possibility of establishing hybrid soybean as a commercial entity have been gained. Understanding heterosis in soybean and identification of general and specific combining ability effects among parents will be necessary and will require extensive studies conducted across a wide range of environments with different sources of male sterility in common and different highyielding genetic backgrounds. Hybrid seed to conduct these experiments can be obtained using insect-mediated pollinations, which has proven to be an efficient method to produce large quantities of hybrid seed. Once this information is collected and positive and economic heterosis estimates are identified, it will be necessary to devise predictive systems to identify genotypes with good combining ability. These studies, and information already collected, will help determine the economics and the feasibility of commercial hybrid soybean as a means to increase seed yield for the grower.

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